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KINETICS AND METABOLISM OF CLOBAZAM IN ANIMALS AND MAN

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1 The pharmacokinetic behaviour of the psychotropic drug clobazam, a 1,5 benzodiazepine, and its metabolism were studied with the ^{14}C-labelled compound in rats, dogs, monkeys and man. The absorption was practically complete in all three animal species. Clobazam was not excreted in the unchanged form by all species. The main metabolite in plasma of monkeys, dogs and man was *N*-demethylclobazam. The metabolites were partially in the conjugated form.

2 The binding to serum proteins (concentration range 0.05–10 μg/ml serum) amounted to between 66% (in rats) and 85% (in man). The maximal levels of total radioactivity (original compound and metabolites) in blood were 0.24 ± 0.043 μg Equ/ml (2–4 h) in doses and 0.67–0.82 μg Equ/ml (0.5–1 h) in rhesus monkeys. These levels were markedly higher than those in rats with values of 0.064 ± 0.012 μg Equ/ml (~0.5 h). The elimination of radioactivity from blood occurred in two phases.

3 After repeated daily administration of oral doses, the 24-h blood levels accumulated in rats to about three times the initial value. In dogs the 24-h serum concentrations remained practically unchanged. Long-term treatment with clobazam in monkeys neither caused enzyme induction nor other processes retarding metabolism and elimination.

4 Both after a single oral and intravenous dose, more than two-thirds of the radioactivity administered to rats was excreted with the *faeces*. Dogs, however, excreted about three-quarters of the radioactivity with the *urine*, irrespective of the route of administration. In monkeys, the excretion also occurred mainly in the urine. In all three species, *renal* excretion was similarly rapid to that from blood or plasma.

5 Apart from gastro-intestinal tract, liver and kidneys, the distribution in rats and dogs was remarkably even within the range of maximal blood levels. In the rat brain, the concentration amounted to only one-third of that in the blood. Special accumulations were not found. In dogs, the concentration in the brain was as high as that in the blood.

6 In rats, kinetics and metabolism were not significantly changed by pregnancy.

7 For metabolism studies in the four species (man, monkey, dog and rat) urine and faeces (and in some cases also serum) were examined after a single dose or repeated administration. The number and kind of metabolites detected in the individual species were partially different. In autoradiographic studies, exceptionally up to 14 radioactive spots were found for clobazam.

8 The structures of the metabolites were elucidated by independent methods, mainly mass spectrometry. In addition to the original substance, eight metabolites were identified for clobazam amounting to 70–90% of the total number of metabolites, depending on the species.

The two most important chemical changes of clobazam during metabolism are dealkylation and hydroxylation. Dealkylation at nitrogen-(1), particularly pronounced in the species dog, does not differ between the 1,4- and 1,5-benzodiazepines. The difference in metabolism is only pronounced in oxidative decomposition.

9 In contrast to diazepam, the 4′ position of the phenyl ring of clobazam seems to be particularly favourable for introduction of a hydroxyl function. In dogs, hydroxylation at the 9 position plays an additional important role. It results in the formation of the metabolite 9-hydroxy-*N*-demethylclobazam by which this species is markedly distinguished from the other three species.

It is remarkable that clobazam is not hydroxylated at the 3-position. This is obviously a characteristic of the 1,5-benzodiazepines and helps to distinguish them from the 1,4-benzodiazepines such as diazepam.

Introduction

CLOBAZAM, a psychotropic drug with the test designation HR376, belongs to the 1,5-benzodiazepines the pharmacological properties of which are largely unknown (Rossi *et al.*, 1969). The 1,5-benzodiazepines differ from the well known 1,4-benzodiazepines, the most famous representative of which is diazepam, only by carbon(C) and nitrogen(N) in the isomeric positions 4 and 5 of the diazepine ring (Figure 1). Although the structural formula of

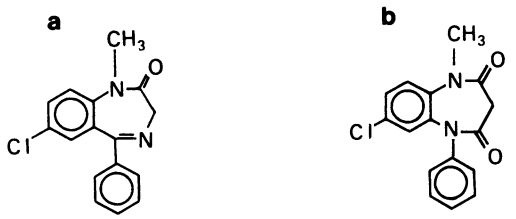

Figure 1 Structural differences between (*a*) diazepam, a 1,4-benzodiazepine and (*b*) clobazam, a 1,5-benzodiazepine.

clobazam differs only slightly from that of diazepam, both compounds exhibit marked distinctions in their chemical reactivity and metabolism.

The pharmacokinetic behaviour of clobazam and its metabolism were studied with the ^{14}C-labelled compound in rats, dogs, monkeys and man (Figure 2). The

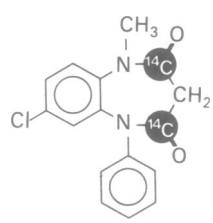

Figure 2 2,4-[^{14}C]-Clobazam.

type of labelling selected proved to be biologically stable; no radioactivity in the form of carbon dioxide was exhaled.

The following is a report on the pharmacokinetics and metabolism of clobazam in rats, dogs and monkeys as well as on metabolism in man. The pharmacokinetics in man will be described elsewhere (Rupp *et al.*, 1979).

Methods

In general, clobazam was administered orally as a starch suspension; dogs received the compound in gelatin capsules. For intravenous injection, the substance was dissolved in saline to which a solubilizer (Tween) had been added. The pharmacokinetic behaviour was examined after a single dose as well as after repeated administration.

Results

In our studies clobazam was not excreted in the unchanged form by any species. Consequently the portion of original substance in the plasma was already small even shortly after administration. The main metabolite in plasma was *N*-demethylclobazam. The metabolites were partially in the conjugated form.

For serum concentrations between 0.05 and 10 μg/ml, the binding to serum proteins (Table 1)

Table 1 Binding of ^{14}C-clobazam to serum proteins

Species	*% Binding*	*Concentration range measured*
Rat	66 ± 2	0.05–10 μg/ml
Dog	83 ± 2	
Monkey	75 ± 3	
Man	85 ± 3	

amounted to 66% in rats, 75% in monkeys, 83% in dogs and 85% in man. The percentages were calculated using the method of equilibrium dialysis (Scholtan, 1962). The radioactivity bound to the formed blood elements was always markedly below that of serum.

After oral administration of the compound in the dose range indicated in Table 2, the absorption was

Table 2 Blood levels after oral administration of ^{14}C-clobazam

Species	*n*	Maximum total concentration (μg/ml)	*Time (h after application)*	*Dose (mg/kg)*
Rat	6	0.046 ± 0.012	~0.5	0.52
Dog	5	0.24 ± 0.043	2–4	0.50
Monkey	2	0.67 ; 0.82	0.5;1	2.5

practically complete in all three animal species. Table 2 also shows maximal blood levels for the total concentration in the animal species examined and the times at which they were reached. Total concentration refers to the original compound and the metabolites.

Dogs

The blood levels in dogs and monkeys were markedly higher than those in rats. In dogs, the total concentration measured from the maximum onwards initially was almost exclusively *N*-demethylclobazam. The formation of this compound and the coincident dis-

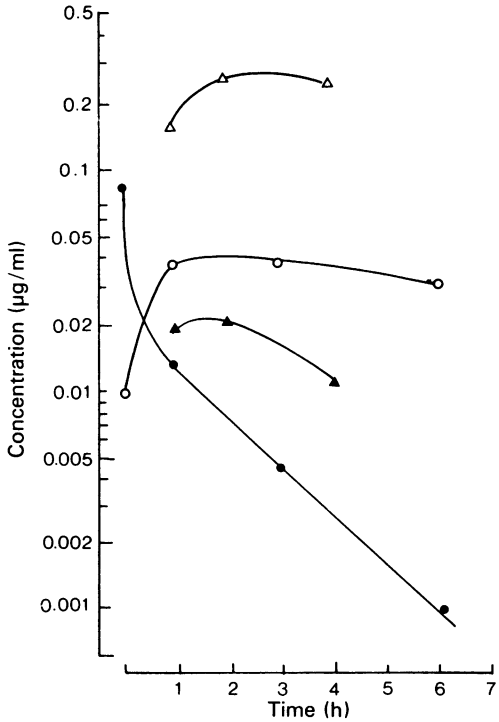

Figure 3 Concentration of extractable clobazam (▲, ●) and *N*-demethylclobazam (△, ○) in plasma after a single oral dose of 0.50 mg/kg (triangles) and a single intravenous dose of 0.10 mg/kg of ^{14}C-clobazam (circles), respectively, to a dog.

appearance of clobazam is shown in Figure 3. The concentrations of *N*-demethylclobazam measured fluorometrically after administration of a single dose of clobazam were practically identical with those measured radiometrically. During 8 weeks of treatment with 2.5 mg/kg, the 24-h serum concentrations remained practically unchanged (Figure 4). Following

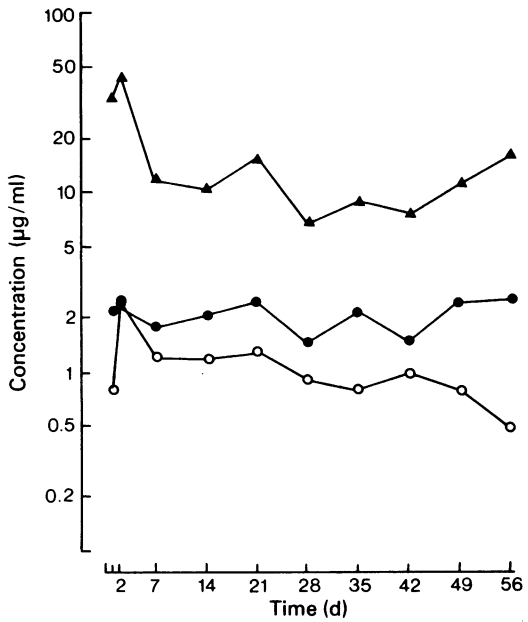

Figure 4 Serum levels of *N*-demethylclobazam during chronic administration of ^{14}C-Clobazam in dog. ▲, H_{16} 40 mg/kg; ●, H_{14} 2.5 mg/kg; ○, H_{18} 2.5 mg/kg.

a dose of 40 mg/kg administered over the same period of time, the serum levels were lowered from the end of the first week onwards. Radiometric measurements revealed a continuous and biexponential decrease of the concentrations from the maximum onwards, with half-lives of 3.2 ± 0.7 h and 32 ± 11 h (figure 5).

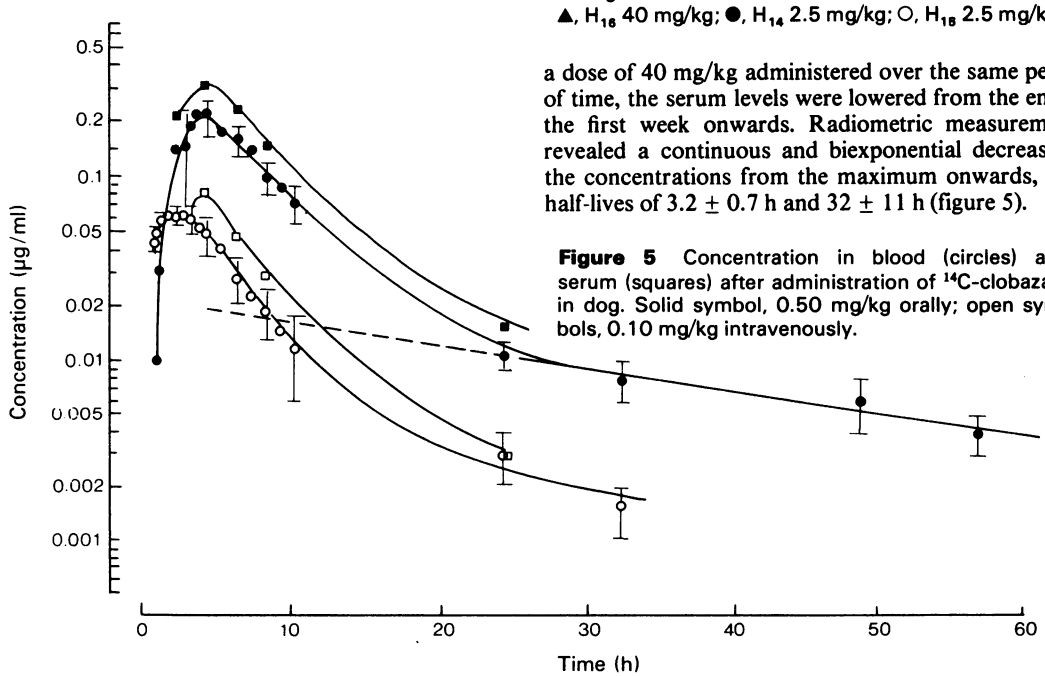

Figure 5 Concentration in blood (circles) and serum (squares) after administration of ^{14}C-clobazam in dog. Solid symbol, 0.50 mg/kg orally; open symbols, 0.10 mg/kg intravenously.

Rats

In rats, clobazam was also very rapidly metabolised. This was revealed by the 0.5-h values and 6-h values both after a single dose and the last of ten oral doses. In the first case only about 4% of the extractable compounds was available in the unchanged form; in the latter case, clobazam had disappeared completely.

Figure 6 shows the course of total concentration in blood following the administration of a single dose – represented by the lower curve *B* on the right – and after ten doses – represented by curve *A*. The retardation of elimination observed after a single oral dose from the maximum onwards resembled that after intravenous administration and might be mainly caused by *N*-demethylclobazam flowing into the central compartment. Between 8 and 32 h after dosing, the concentration decreased with a half-life of about 7 hours.

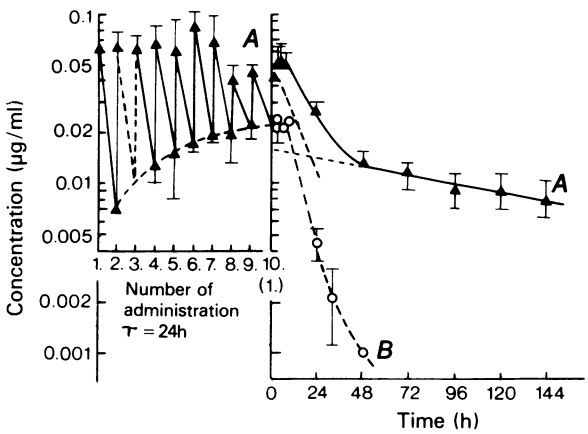

Figure 6 Concentrations in rat blood after a single dose and during repetitive dosing of ^{14}C-clobazam.

After repeated daily administration of oral doses, the 24-h values cumulated to about three times the initial value. Thus, the steady state minimum seemed to have been reached. The cumulation was mainly induced by the slow elimination process which was hardly detectable after a single dose, and pronounced after administration of ten doses, as can be seen from the elimination portion of curve *A*. Elimination occurred in two phases with half-lives of 12 ± 4 h and 121 ± 22 hours.

Monkeys

In monkeys, we also examined whether long-term medication with clobazam had an effect on the kinetic parameters. Initially, the animals received a single oral dose of ^{14}C-clobazam 2.5 or 20 mg/kg body weight. After determination of the pharmacokinetic parameters, the monkeys were treated with the same dose of the unlabelled compound over 4 weeks. Finally the radioactive compound was once more administered in the previous dose. The blood levels measured are shown in Figure 7. The degree of absorption did not depend on the dose. However, as a result of the filling up of peripheral compartments during long-term medication, the blood levels were markedly higher after the second radioactive dose than after the first. The half-lives found for the concentration decrease after the two labelled doses were dose-dependent, but were identical within a dosage group.

These findings permit the conclusion that long-term treatment with clobazam in monkeys neither caused enzyme induction nor other processes retarding metabolism and elimination.

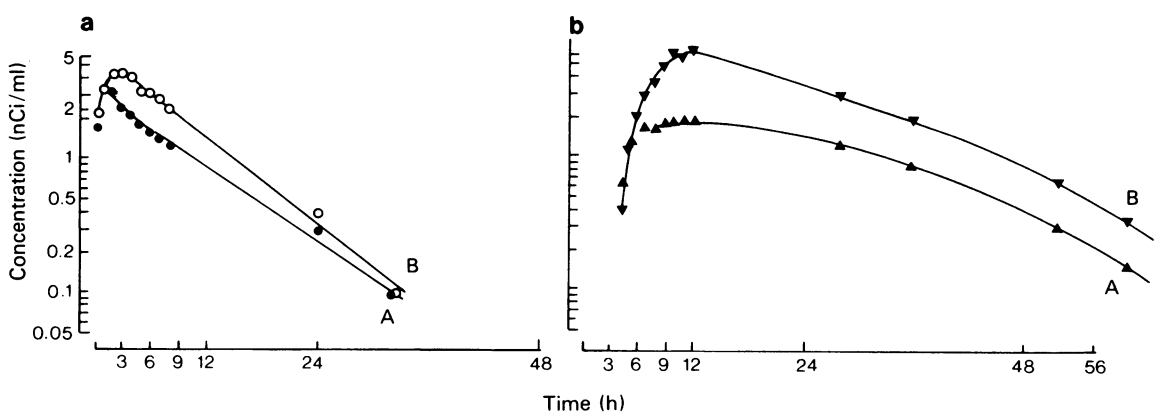

Figure 7 Concentration in monkey blood after oral administration of 2.5 and 20 mg/kg clobazam respectively. *a*, Monkey 2, 2.5 mg/kg: ○, t_{50} = 5.5 h; ●, t_{50} = 6.5 h. *b*, monkey 3, 20 mg/kg: ▾, t_{50} = 10.1 h; ▲, t_{50} 10.4 h. *A*, First radioactive dose at beginning of trial; *B*, second radioactive dose after a 4-week treatment with non-radioactive clobazam.

Excretory routes

Table 3 demonstrates the excretory routes after administration of ^{14}C-clobazam. Both after a single oral and intravenous dose, more than two-thirds of the radioactivity administered to rats was excreted with the faeces. Dogs, however, excreted about three-quarters of the radioactivity with the urine, irrespective of the route of administration. In monkeys, the excretion also occurred mainly in the urine. After administration of 10 oral doses of clobazam to rats, the portions of the total dose recovered in the urine were about a quarter lower than after administration of a single dose. However, this difference was already observed after administration of the first dose, so that this finding can obviously not be attributed to long-term medication.

In all three species, renal excretion was just as rapid as that from blood or plasma.

Distribution in tissues

Apart from the gastro-intestinal tract, liver and kidneys, the distribution in rats and dogs was remarkably even, within the range of maximal blood levels, as can be seen from the whole body autoradiogram (Ullberg, 1954) of a rat (Figure 8). In the rat brain, the concentration amounted to only one-third of that in the blood. Accumulations were not found. In dogs, the concentration in the brain was as high as that in the blood.

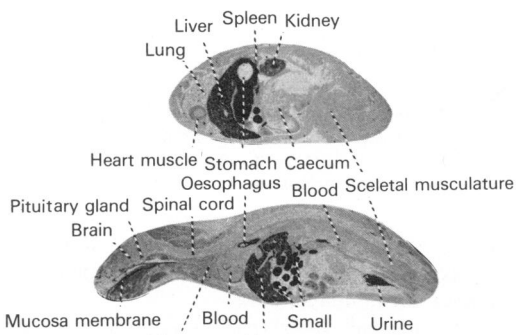

Figure 8 Whole-body autoradiogram of a rat 0.5 h after oral administration of ^{14}C-clobazam.

Pregnancy

In rats, additional studies on pharmacokinetics and metabolism in pregnancy were performed. As the autoradiograms of the urine of pregnant and non-pregnant animals reveal (Figure 9), the metabolite

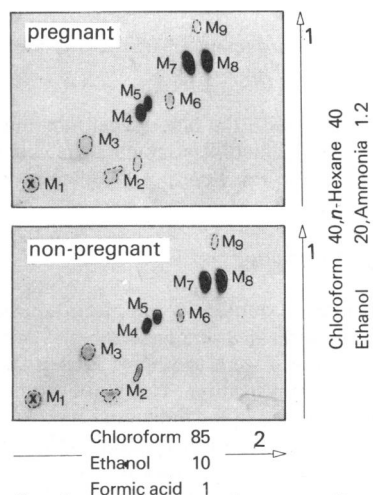

Figure 9 Autoradiogram of a two-dimensional thin-layer chromatogram of rat urine (0–24 h) after administration of a single dose of ^{14}C-clobazam 0.5 mg/kg orally to a pregnant and a non pregnant female rat.

patterns were identical. Both pregnant and non-pregnant rats excreted almost exclusively metabolites. We will refer later to the identification of the individual spots. The distribution in the mother animal and the foetus (Figure 10) was again remarkably even, if the excretory organs of the dam are not considered. The

Table 3 Excretion after administration of ^{14}C-clobazam to different animal species

Species	*Route of administration*	*Dose (mg/kg)*	*Excretion (% administered dose)*		
			Urine	*Faeces*	*Balance*
Rat	Intravenously	0.10	27 ± 1	73 ± 6	100 ± 6
Rat	Orally	0.52	29 ± 6	71 ± 7	100 ± 1
[Rat	*Orally 10×*	0.57	21 ± 3]	*	—
Dog	Intravenously	0.10	78 ± 9	28 ± 4	106 ± 2
Dog	Orally	0.50	74 ± 5	28 ± 2	102 ± 3
Monkey	Orally	2.5	61 ± 14	*	—

*Not determined.

selective enlargement (Figure 11) demonstrates that less than 1% of the dose administered to the dam was contained in the concentration maximum of the foetus,

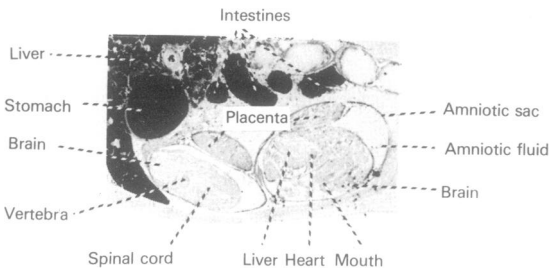

Figure 11 Enlargement of uterus and foetuses of the boxed area in Figure 10.

which coincided with the blood level maximum of the mother animal. The studies indicate that kinetics and metabolism in the rat were not significantly changed by pregnancy.

Metabolite distribution

For metabolism studies in the four species man, monkey, dog and rat, urine and faeces – and in some cases also serum – were examined after a single dose or repeated administration. The number and kind of metabolites detected in the individual species were partially different. In autoradiographic studies, exceptionally up to 14 radioactive spots were found for clobazam. However, a number of them were so slight that they could not be quantitatively determined and identified. Nevertheless, we believe that we succeeded in elucidating the principles of the metabolism of clobazam. In addition to the original substance, eight metabolites were identified for clobazam amounting to between 70 and 90% of the total number of metabolites, depending on the species.

The structures of the metabolites were elucidated by independent methods, mainly mass spectrometry. As far as reference compounds were available, which applied to the majority of metabolites, mass spectrometric findings were verified by thin-layer chromatography. Moreover, the identity of a metabolite was demonstrated by isotope dilution in all cases in which appropriate precipitation methods had been found. If mass spectrometry did not suffice for metabolite structure elucidation, nuclear magnetic resonance spectrometry (NMR) or appropriate derivatization was applied additionally to convert the metabolite into a known reference compound.

After oral administration of clobazam, the individual species showed the following metabolite distribution:

Dog (Figure 12). Here is the *autoradiogram* of a urine sample separated by two-dimensional thin-layer

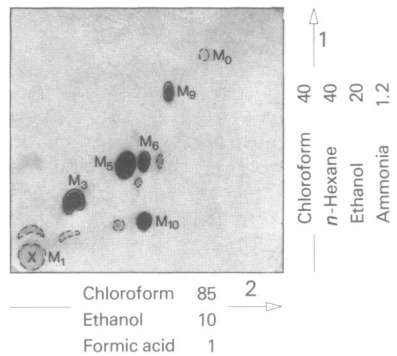

Figure 12 Autoradiogram of a two-dimensional thin-layer chromatogram after administration of ^{14}C-clobazam 40 mg/kg to a dog. Repeated doses orally. Urine: 0–24 h after dose digested with β-glucuronidase/arylsulphatase.

chromatography. The black spots on the X-ray film show exactly where radioactive metabolites are situated on the thin-layer plate.

In the dog, all metabolites found in urine and faeces were derived from *N*-demethylclobazam. *N*-

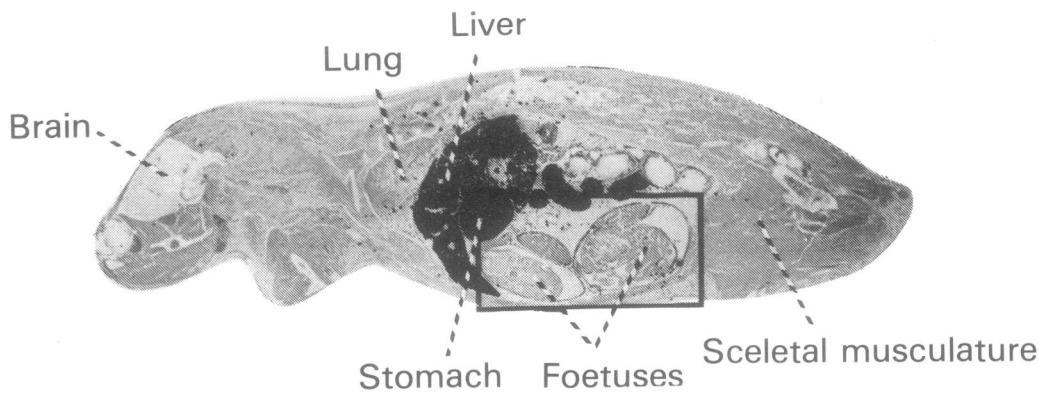

Figure 10 Whole-body autoradiogram of a pregnant rat 2 h after oral administration of ^{14}C-clobazam.

dealkylation was very pronounced in this species and obviously occurred very rapidly. Only about 2 h after administration, practically all metabolites detectable in the urine were *N*-demethylclobazam derivatives. Independent of the dosage and number of doses – the compound was given in oral doses of 2.5 or 40 mg/kg both as a single dose and a daily dose over three months – the following seven substances were found in urine as well as in faeces: traces of M_0, the original substance; M_1, an unknown metabolite located near the starting point; M_3 (*N*-demethylclobazam-3′, 4′-dihydrodiol); M_5 (= 4′-hydroxy-*N*-demethylclobazam); M_6 (= (3′, 4′)-hydroxymethoxy-*N*-demethylclobazam); M_9 (= *N*-demethylclobazam); and M_{10} (= 9-hydroxy-*N*-demethylclobazam). M_{10} is a metabolite specific to dogs which was not encountered in the other species examined, or only found in traces. This metabolite was identified by mass spectrometry and NMR spectroscopy, and its structure was confirmed by synthesis (Reid, W. & Sell, G., unpublished). The main metabolite in dogs was M_5 (= 4′-hydroxy-*N*-demethylclobazam). The major part of these metabolites was found in urine as glucuronide. As mentioned above, the same metabolites were found after a single dose and repeated administration; the quantitative composition also revealed no difference.

Monkey (Figure 13). Male and female rhesus monkeys received clobazam in oral doses of 2.5 or 20 mg/kg

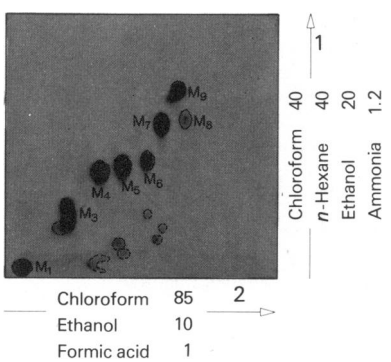

Figure 13 Autoradiogram of a two-dimensional thin-layer chromatogram after administration of ^{14}C-clobazam 2.5 mg/kg to monkey. Repeated doses orally. Urine: 0–24 h after dose digested with β-glucuronidase/arylsulphatase.

both as a single dose and as a daily dose over 4 weeks. In addition to the sporadically observed original substance M_0, the unidentified metabolite M_1 as well as metabolites M_3 to M_9 were found: the dihydrodiols of clobazam (M_4) and *N*-demethylclobazam (M_3), the phenols of clobazam (M_7) and *N*-demethylclobazam (M_5) hydroxylated at the 4′ position, the hydroxymethoxy compounds of clobazam (M_8) and *N*-demethylclobazam (M_6), and *N*-demethylclobazam itself (M_9). M_{10}, the 9-hydroxy product of *N*-demethylclobazam, could not be detected. As in dogs, the main metabolite in monkeys was M_5. All metabolites were found almost quantitatively in the conjugated form as glucuronide and sulphate.

The metabolism revealed neither a dependence on the dose nor on sex. However, a quantitative (nor qualitative) difference was observed between a single dose and repeated administration in the urine and faeces. The formation of *N*-demethyl derivatives seemed to be favoured by repeated administration.

Rat (Figure 14). The urine of rats which had been given a single oral dose of clobazam 20 mg/kg showed

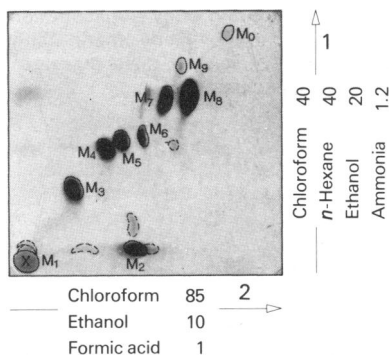

Figure 14 Autoradiogram of a two-dimensional thin-layer chromatogram after administration of ^{14}C-clobazam 20 mg/kg to rat. Single dose orally. Urine: 0–24 h after dose digested with β-glucuronidase/arylsulphatase.

– as in monkeys – the substances M_3 to M_9. The unidentified metabolites M_1 and M_2 were quantitatively insignificant. M_0, the original substance, and M_{10} were not detected in the urine. In the faeces, in addition to M_0 and the unknown substance M_1, the metabolites M_4 to M_8 occurred. Two further metabolites, the constitution of which has not yet been elucidated, were detected exclusively in the rat faeces. The main metabolite in rats was M_8 (3′,4′)-hydroxymethoxyclobazam). The rat metabolites in the urine were mainly excreted as sulphates.

Man After a single oral administration of clobazam 38 mg per test person, the serum was examined. Mainly clobazam (M_9) and the metabolites *N*-demethylclobazam (M_9) and 4′-hydroxyclobazam (M_7) were found, with sporadically some 4′-hydroxy-*N*-demethylclobazam (M_5). In the urine (Figure 15), all metabolites from M_1 to M_9 with the exception of M_{10} as well as the original substance were present. The faeces showed a similar pattern. In addition to M_{10}, metabolites M_6 and M_8 were also missing. The main metabolite in man was initially M_7. Later on (from about 5 d after administration onwards) a shift towards M_5 was observed.

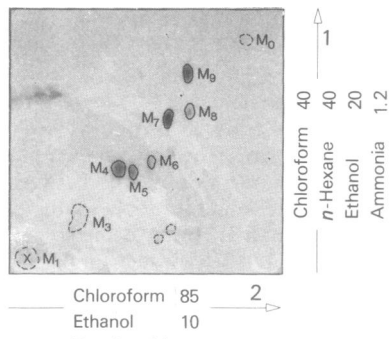

Figure 15 Autoradiogram of a two-dimensional thin-layer chromatogram after administration of ^{14}C-clobazam 39 mg per subject (man). Single dose orally. Urine: 12–24 h after dose digested with β-glucuronidase/arylsulphatase.

All species

The following scheme shows the metabolites of clobazam detected in the individual species (Figure 16). As can be seen, the compounds are divided into two series: on the left side are all metabolites, including the original substance M_0, which are derived directly from the structure methylated at nitrogen-(1), on the right side are all metabolites derived from M_9, the *N*-demethylated form of clobazam. By hydroxylation at 4′-position, the phenolic metabolites M_7, (4′-hydroxyclobazam) and M_5 (4′-hydroxy-*N*-demethylclobazam) which is the *N*-demethylated form of M_7, are formed. They represent the main metabolites in man, monkeys and dogs. Hydroxylation at 9 position results in metabolite M_{10} (9-hydroxy-*N*-demethylclobazam). This metabolite is specific to the species dog and is not found (or, if so, only in traces) in the

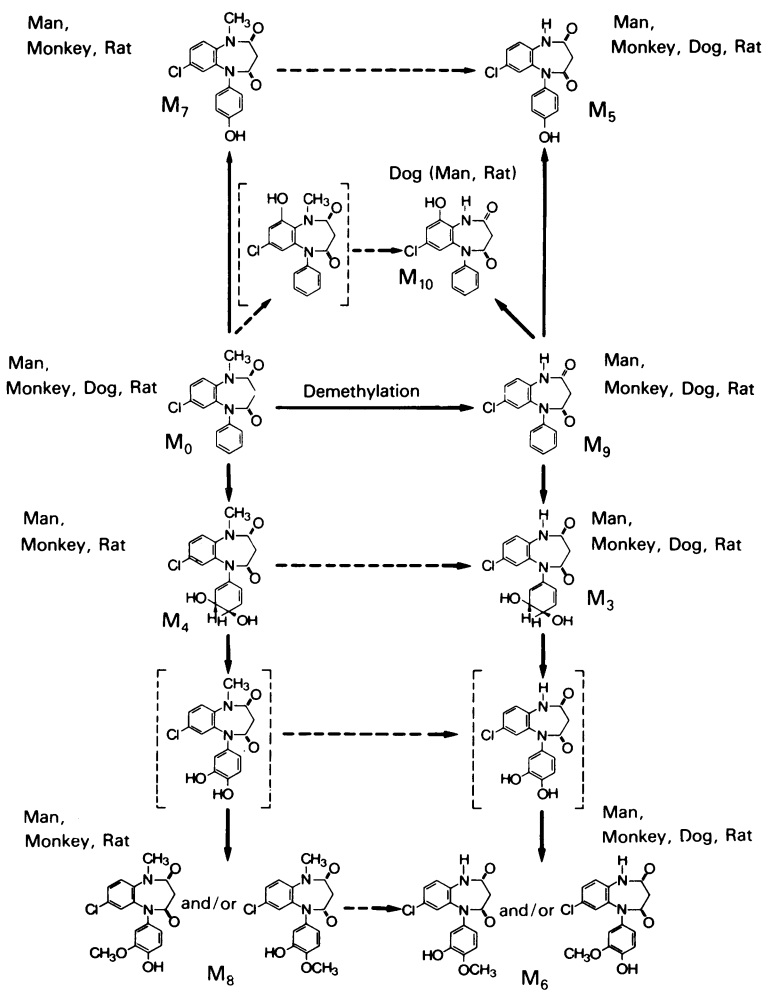

Figure 16 Metabolism of clobazam in man, monkey, dog and rat.

other species. A metabolite which corresponds to M_{10} but shows a methyl group at nitrogen – in broken square brackets – has not yet been detected.

The two dihydrodiols M_3 and M_4 have probably been formed from the corresponding epoxides by hydration (Oesch, 1973). The diphenols – in broken square brackets – formed of M_3 and M_4 by oxidation have not yet been detected as metabolites, but the corresponding monomethyl ethers M_6 and M_8 have. Whether only one of the two possible isomers or the isomeric mixture is present, could not be ascertained.

As we have seen, the two most important chemical changes which clobazam undergoes during metabolism are dealkylation and hydroxylation. Dealkylation at nitrogen-1, which is particularly pronounced in the species dog, does not differ between the 1,4- and 1,5-benzodiazepines (De Silva *et al.*, 1964; Jommi *et al.*, 1964). The difference in metabolism is only pronounced in oxidative decomposition.

Man, dog and monkey hydroxylate 1,4 benzodiazepines – the prototype of which is considered to be diazepam – almost exclusively at the 3 position of the heterocyclic ring (Jommi *et al.*, 1964; Swartz *et al.*, 1965), whereas in the rat the 4′ position of the phenyl ring prevails over the 3 position (Swartz *et al.*, 1967). This observation was also made with other 1,4-benzodiazepines and seems to be a peculiarity of this species (Volz, M. unpublished).

In contrast to diazepam, the 4′ position of the phenyl ring of clobazam seems to be particularly favourable for introduction of a hydroxyl function, because the corresponding 4′-hydroxy metabolites occur not only in rat but also in man, dog and monkey. In dogs, hydroxylation at the 9 position plays an additional important role. It results in the formation

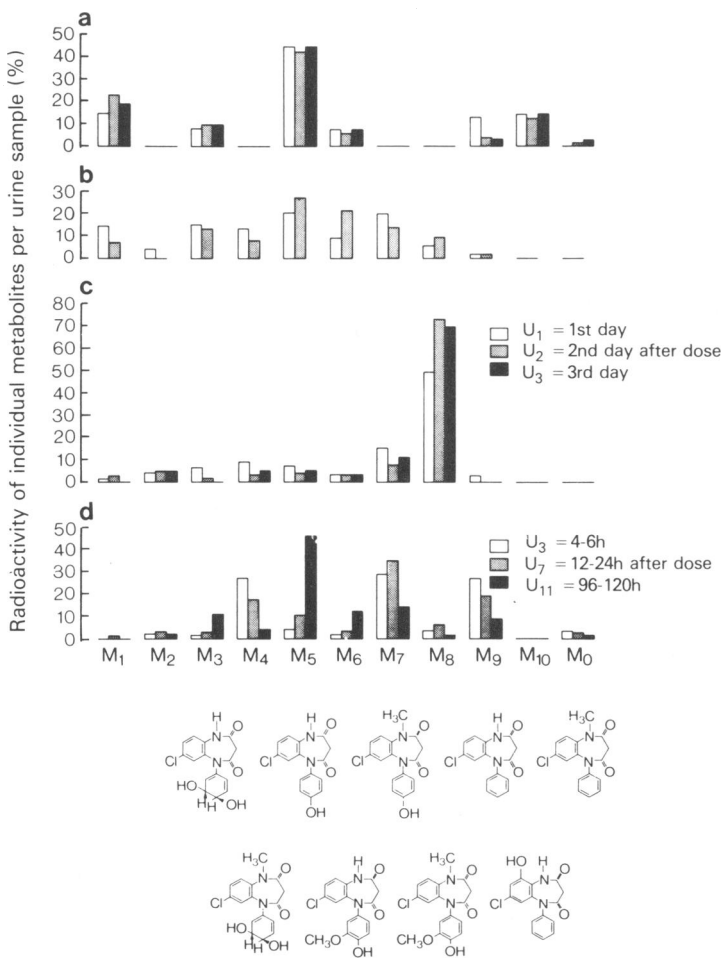

Figure 17 Graphic presentation of the metabolite distribution in the urine of the individual species on the basis of the measured values. *a,* Dog (single dose of 2.5 mg/kg orally); *b,* monkey (single dose of 2.5 mg/kg orally); *c,* rat (single dose of 20 mg/kg orally); *d,* man (single dose of 39 mg per subject orally).

of the metabolite 9-hydroxy-*N*-demethylclobazam by which this species is markedly distinguished from the other three species.

It is remarkable that clobazam is not hydroxylated at the 3 position. This is obviously a characteristic of the 1,5-benzodiazepines and helps to distinguish them from the 1,4-benzodiazepines. In this connection it is noticeable that another 1,5-benzodiazepine, triflubazam, which carries a trifluoromethyl group where clobazam has a chlorine atom, is also not hydroxylated at the 3 position (Grimes *et al.*, 1973; Alton *et al.*, 1975).

In the development of a drug, the choice of an animal model representative of man plays an important role. Pharmacological and toxicological studies should be carried out in that animal species which is closest to man in the metabolism of this specific substance. The example of clobazam demonstrates how difficult it sometimes is to find such an animal species. Although the metabolism in rats and monkeys is qualitatively similar to that in man, there are sometimes pronounced quantitative differences (Figure 17). This shows a comparison of quantitative metabolite distribution in the urine of dogs, monkeys, rats and man at different times after oral administration. As the half-lives of metabolite M_9 (*N*-demethylclobazam) and its derivatives are relatively long, we selected such a late time interval as the fifth day for urine examination in man in order to be able to make a complete statement on the kind and amount of metabolites.

Numerous metabolism studies of 1,4-benzodiazepines have shown that the dog is a very suitable animal model for man in this substance class; however, this species is inappropriate for studies with 1,5-benzodiazepines like clobazam. The rat is irrelevant as an animal model for man in studies with 1,4-benzodiazepines and only conditionally appropriate for studies with 1,5-benzodiazepines. Although the majority of metabolites occurring in man also appear in rat, quantitative differences are pronounced, particularly in metabolite M_8 ((3',4')-hydroxymethoxyclobazam).

Even the rhesus monkey, in our studies the species phylogenetically next to man, is not always the most appropriate animal model for man. This is clearly demonstrated by the significant differences between the distribution of M_1, M_3 and M_9. Thus, we have frequently no other choice than to carry out a combined study using several animal species.

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